THE EFFECTS OF MONOCULAR DEPRIVATION
ON THE CYTOARCHITECTURE
OF THE DEVELOPING KITTEN CORTEX

by
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DEDICATION

This is dedicated to Susan who has loved and tolerated me. Now we can enjoy sunsets, pastures, horses and a Grand Mariner with coffee. Thank you Sam.

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Abstract of Dissertation Presented to the
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June, 1972

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Major Department: Psychology

The problem of periods of susceptibility for cortical change (areas 17, 18, 19) in kittens following monocular deprivation was investigated using the Ramon-Moliner ('64) modification of the Golgi-Cox stain and the cresyl violet stain. In all instances it was found that suture of the right eyelid before 20 days post-partum had no detrimental effects. After 20 days however, the longer the deprivation period the greater the difference between deprived and normal control animals. An analysis of stellate cells in layer IV showed that there was greater dendritic branching in the control kittens than in the occluded kittens. Counts of spines on pyramidal cell apical dendrites, which passed through layer IV, revealed a greater spine density in the

nondeprived animals. The superiority of the normal animals was also reflected in increased cortical thickness as compared to the experimental kittens; however no difference between the groups was found with regard to volume of nuclei. These data indicate that until day 20 neuronal development is mainly under genotypic control but after this period environmental manipulation can affect cell morphology or phenotype. One interesting finding not related to deprivation but which may indicate a type of hemisphere dominance was that the number of spines on basal dendrites in area 17 was greater in the left hemisphere than in the right. This occurred regardless of environmental manipulation.

INTRODUCTION

Roux (1895) suggested that the mammalian nervous system undergoes two stages of development: (a) an early stage of self differentiation; (b) a postnatal period of growth depending on functional activity. The basic components of the nervous system are genotypically determined while their phenotypic expression is, to a large measure, dependent on environmental experiences.

If the second part of this hypothesis is valid then the structure of a neuron can be altered by environmental manipulation resulting in a phenotypically different cell. One of the methods for testing this is to deprive a developing unit in the nervous system of environmental contact and observe the consequences. Because of its great importance and relative ease of manipulation, the visual system has been a major subject of deprivation studies over the past two decades. All of the major centers along the visual pathway have been investigated either biochemically, histologically, electrophysiologically or behaviorally.

Since the retina is the initial receptor site it was one of the first centers to be studied. Brättgard ('52) raised newborn rabbits in total darkness until they were ten weeks old and then assayed for nucleoprotein in ganglion cells. Dark-reared animals had significantly less ganglion cell nucleoprotein than normals. Brättgard concluded that, "The cell is dependent on adequate stimulation for development of proteins. If it is deprived of stimulation, production is reduced or inhibited."

Structural changes have also been observed in other areas of the retina following deprivation. Weiskrantz ('58) raised kittens in 23 hours of daily darkness for 17 weeks. During the remaining one hour a day the animals received diffuse illumination in one eye and normal illumination in the other eye. The result was a 20 per cent reduction in Müller fibers and thinning of the inner plexiform layer in both eyes.

Reisen ('60) and Rasch et al. ('61) compared the effects of normal light and total darkness of young carnivores and primates. In the cat the retina developed with a thinner inner plexiform layer and reduced RNA in ganglion, bipolar and receptor cells. Chimpanzees also showed this RNA decrement, but in contrast to the cats they also had a 90 per cent loss of ganglion cells. With the electron microscope Cragg ('69) found, in light-deprived rat retinae, enlarged synaptic terminals in the outer plexiform layer.

Although the synapses were larger in light-deprived eyes there was no difference in the thickness of any retinal layer.

Although cats and mice do not appear to lose ganglion cells during light deprivation the number of optic tract fibers is decreased and the remaining fibers show less myelinization (Weiskrantz, '58; Gyllenstein and Malmfors, '63). However, studies by Terry, Roland and Rose ('62) and Gyllenstein, Malmfors and Norrlin ('66) found no difference in the optic tract fibers between dark-reared and normal young mice. Gyllenstein _et al._ ('66) explained this disparity by pointing out that in the latter experiments animals were light-deprived two months longer. A type of normalization might have taken place in the longer time period of deprivation. In the light of results which will be presented in the body of this paper, this explanation appears to be valid.

The first sensory deprivation studies on the subcortical visual centers used the method of eye enucleation and showed transneuronal degeneration in the lateral geniculate nucleus (LGN) (Cook, Walker and Barr, '51; Hess, '58). The dependence of a post-synaptic cell's integrity on the presence of pre-synaptic innervation is not hard to understand since transneuronal degeneration is a well known neuroanatomical phenomenon. Brandes ('71) confirmed the earlier findings of nuclear shrinkage but noted that dendritic branching was not affected.

It was not until the 1960s that transneuronal effects of sensory deprivation in the intact visual system were carefully studied. Gyllenstein, Malmfors and Norrlin ('65, '68) reared young mice in total darkness from birth to 24 months. Their results showed a decrease in neuronal cell body diameter and a decrease in internuclear mass in both the LGN and superior colliculus. Assays of proteins, choline acetyltransferase and acetylcholine esterase in the LGN and superior colliculus of dark-reared rats (21 days) showed significant decreases in these compounds as compared with normals (Maletta and Timeris, '67, '68).

In cats raised with one eye occluded from birth there was a 40 per cent decrease of perikaryon size in the LGN cells receiving input from the deprived eye (Hubel and Weisel, '63). Monocularly occluded rats show similar effects in LGN, but unlike cats the effects could be produced when the occlusion occurred in adulthood as well as right after birth (Fifkova, '67; Fifkova, Hassler, '69; Fifkova, '70). Since Cragg ('69) found a decrease in synaptic density in the LGN cells of rats raised in the dark from birth, it would appear that binocular or monocular light deprivation results in morphological changes in LGN. Eysel, Flynn and Gaerdt ('71) found electrophysiological effects in LGN in monocularly deprived kittens. They found that the inhibition in center-surround cells was not as strong in cells innervated by the deprived eye.

The most interesting research on the effects of the environment on the visual system has been concerned with the visual cortex. Rosensweig, Krech, Bennett and Diamond ('62) compared a group of young rats raised in an enriched environment (ECT) with rats raised in an impoverished environment (ICT). The parameter measured was the total amount of cholinesterase in visual cortex. Rats in the ICT group had significantly less cholinesterase than the ECT group. In addition, deficits were found in the ICT group with regard to thickness of visual cortex (Diamond, Krech and Rosensweig, '64); glial/neuron ratio (Diamond, Law, Rhodes, Lindner, Rosensweig, Krech and Bennett, '66); nuclear size of neuron (Diamond, '67); and dry brain weight (Bennett, Rosensweig and Diamond, '69). However, the ICT group showed in increase over the ECT group in the number of neurons per area of microscopic field. Rosensweig ('66) offered the explanation that the ECT group had greater ramification of processes, causing the number of nuclei per field to be decreased. The greater branching plus the glial proliferation and increase in neuron nuclear size accounts for the increase in cortical thickness. Holloway ('66) used a Golgi stain on a similar group of ECT and ICT rats and confirmed Rosensweig's hypothesis that dendritic branching is affected by environmental conditions, i.e., the ECT group had larger dendritic fields. In an electron microscopic study of the bouton termineaux in the visual cortex of ICT and ECT rats, Greenough, West and Fleichman ('71) found that ECT animals had a larger contact area per synapse as indicated by increased length of post-synaptic bars.

Fifkova ('68, '70) showed that monocularly deprived rats had an 8 per cent increase in thickness of visual cortex. Neurochemical changes have been found in chickens following monocular deprivation and enucleation (Bondy and Margolis, '70). Enucleation or monocular deprivation in the chicks causes a reduction in the rate of protein and RNA synthesis in the cerebral hemispheres and optic lobes.

The use of the Golgi stain has brought to light one of the most interesting results of the visual deprivation studies, namely the loss of dendritic spines. Dendritic spines or gemmules are small processes which protrude at a 45 degree angle from the dendritic branches of neurons. These processes were first described by Ramon y Cajal (1891). The dendritic spines usually have small knobs on the end and normally there are about 0.9 spines per μ of apical dendrite (Valverde, '67). Although the apical dendrites of pyramidal cells have the greatest complement of spines, some may also be found on the basal dendrites and the dendrites of stellate cells. Ramon y Cajal (1896) postulated that the spines increased the receptive surface of dendrites and functioned as synaptic sites. Gray ('59) used the electron microscope to provide proof for Ramon y Cajal's hypothesis. It is still a matter of controversy whether the synaptic spines on the pyramidal cell apical dendrites receive their synaptic projections directly from primary afferents or from the stellates in layer IV. Valverde ('68) believes that both inputs exist. However, Globus and Scheibel ('67) contend that most of the afferent input goes directly to the primary apical dendrite.

Loss of dendritic spines was first investigated by Colonnier ('64). He undercut the cortex in a cat and found not only axonal degeneration, but also post-terminal cytoplasmic loss. On further examination it was found that animals with an undercut cortex had fewer apical pyramidal spines than normals. Colonnier reasoned that the post-synaptic cytoplasmic mass that was lost consisted of dendritic spines. This provides evidence for a type of transneuronal degeneration of synaptic spines in the cortex.

The experiment by Colonnier, however, involved only one or possibly two synapses. Globus and Scheibel ('66) took a more peripheral approach and removed the eyes of newborn rabbits. Counts of apical dendritic spines of pyramidal cells in the visual cortex revealed that there were significantly fewer spines in the enucleated rabbits than in normals. The loss occurred only on the main branch and not on the oblique branches or on basal dendrites. These same investigators tested the effects of total light deprivation on the intact rabbit and found that the number of spines did not decrease, but that the spines in the experimental animals lost their end knobs and were abberant (Globus and Scheibel, '66).

Valverde ('67, '68) and Ruiz-Marcos and Valverde ('69) compared the effects of deprivation and enucleation in young mice. Their findings with enucleation were similar to those of Globus and Scheibel ('66), but different results were obtained with light deprivation. Instead of deformed spines, Valverde found significant spine loss. Fifkova's ('70) findings agreed with Valverde in that occlusion, even though only monocular, caused a 28 per cent decrease in spines after 30 days of light deprivation starting ten days after birth.

Along with spine loss during enucleation and deprivation, Valverde ('67) also found that stellate cells were capable of movement. Upon enucleation, stellate cells migrated out of layer IV in the striate cortex and went to either layer III or V. Valverde said, "It was as if stellate cells which lost their innervation were seeking new synaptic contacts." Enucleation also has an effect on prestriate cortex. Valverde and Esteban ('68) examined the number of spines on pyramidal cells of prestriate cortex both ipsilateral and contralateral to the eye removed, and much to their amazement, found an increase in the number of spines in the cortex ipsilateral to the enucleation. They could offer no explanation for this unique finding.

In addition to the biochemical and histological data on the effects of visual deprivation on the cortex, there are a number of studies which show electrophysiological effects. Weisel and Hubel ('63) used monocular eyelid closure to study the effects of light deprivation in kittens. Their results showed that instead of the normal 80 per cent level of binocularly driven cells in the striate cortex, only 20 per cent of the cells could be binocularly driven after monocular eyelid closure. When the deprived eye was stimulated very few cells could be made to respond in any manner whatsoever. Although the single cell recordings from the cortex were abnormal, the electroretinogram was the same as that of control animals. Upon histological examination of parikaryon diameters the cortex appeared normal. There was no deficit in the light reflex upon stimulating the deprived eye, but the animal was behaviorally blind in that eye.

If occlusion of one eye caused all the above effects, it would appear to follow that bilateral occlusion would result in a greater deficit. Electrophysiologically this was not the case since bilateral occlusion resulted in an almost normal number of cells driven by both eyes (Weisel and Hubel, '65). Over 50 per cent of the bilaterally driven cells had normal receptive fields. This fairly normal cortical activity occurred despite the fact that all the layers of the LGN showed a 40 per cent decrease in cell size from normals and that all animals were behaviorally "blind". Ganz, Fitch and Satterberg ('68) found similar results concerning bilateral versus unilateral occlusion in cats, but with a few interesting exceptions. Whereas Hubel and Weisel found only a few cells that could be driven from the deprived eye, Ganz et al. found many cells which could be driven by that eye. The neurons which were excited by the deprived eye, however, had unusually large receptive fields and had decreased direction and orientation specificity. The authors stated, "The lack of pattern vision in one eye of a young kitten induces a selective and rather permanent change in the connections of visual radiation fibers onto cortical cells." The permanency of this change was demonstrated behaviorally by Ganz and Fitch ('68) who found irreversible deficits in form discrimination when the deprived eye of kittens was tested.

In a somewhat different approach to visual deprivation, Hirsch and Spinelli ('70) and Blakemore and Cooper ('70) deprived kittens of either vertically or horizontally oriented stimuli. Electrophysiological recordings showed that the animals raised without horizontals had few cells which would discharge to stimuli oriented in that direction. The reverse was true of cats deprived of vertically oriented visual stimuli. Behavioral testing confirmed the single cell data. Related to this approach is the work of Shlaer ('71). He deprived kittens of their normal binocular disparity and imposed a new one by means of prisms. This resulted in an increase in stimulus disparity to which a cortical binocularly driven cell would maximally fire.

The effects of deprivation in the intact system and also enucleation have a time dependency. Valverde ('68) found that spine loss was much greater in animals 24 days after enucleation than at 48 days later. Gyllenstein, Malfors and Norrlin ('65, '68) also observed this normalization effect in mice after light deprivation; the decrease in nuclear diameter of cortical neurons was greater at two months of light deprivation than at four months.

Weisel and Hubel ('65), Hubel and Weisel ('70) and Dews and Weisel ('70) demonstrated that in cats the period of cortical neuronal susceptibility is time dependent. According to their work neuronal development in the kitten is genetically determined during the period from birth to 21-23 days since development of neuronal pattern is normal even after severe light deprivation. This seems plausible in light of the work of Purpura, Shofer, Housepain and Noback

('64); Rose and Lindsley ('65) and Huttenlocker ('67). These investigators found that the cortex of a kitten achieves its adult electrical activity in the same 21-23 day time period which is also the time when placing and following responses mature and acuity reaches an asymptote. Hubel and Weisel ('70) and Dews and Weisel ('70) placed the upper limits of susceptability at about eight or nine weeks after birth. After this time visual deprivation in cats had little effect. In addition, behavioral testing in Rhesus monkeys demonstrated that monocular deprivation is only effective at infancy (Von Noorden, Dowling and Ferguson, '71). In contrast to these studies Fifkova ('71) found that both LGN and cortex in rats could be affected even if the unilateral occlusion occurred at 60 days post-natally.

After reviewing the literature it appears that most morphological work in deprivation studies has been done on rodents while most behavioral and electrophysiological data has come from cats. The present study was undertaken to determine the morphological basis of the extensive neurophysiological and behavioral changes following light deprivation in cats. Kittens were monocularly occluded for varying periods of time and the morphological effects of this type of visual deprivation was studied in primary visual cortex and visual association areas.

MATERIAL AND METHODS

The subjects were 20 kittens who were housed in 2x2x2 feet wire cages. The cages were made of one-half inch wire screening to allow ample entry of light and a view of the housing room. The walls of the housing room were painted white on three sides with the fourth wall being natural wood. There were numerous objects about the room; desk, chairs, shelving, etc. The light level in the cages was at 1.4 foot lamberts.

Experimental Paradigm

Littermates were randomly assigned to one of three groups for one of five time periods (Table 1).

The five time periods were either: (a) birth to 20 days post-partum (20 day category), (b) 20 to 30 days post-partum (30 day category), (c) 20 to 40 days post-partum (40 day category), (d) 20 to 50 days post-partum (50 day category) or (e) 20 to 60 days post-partum (60 day category). Kittens in Group I (occluded group, N=10) had their right eyelid sutured for one of these five assigned periods and were immediately sacrificed. Group II (reversal group, N=5) kittens had their right eyelid sutured for one of the five time periods and then instead of being sacrificed the right eyelid was surgically opened and the left eyelid closed. The left eye remained occluded for an additional 30 days whereupon the animals were sacrificed. The Group III (control, N=5) kittens were normal controls and one was sacrificed at the end of each time period along with their Group I counterpart (Table 1). The varying periods of deprivation were chosen to coincide with the electrophysiological data (Hubel and Weisel, '70) and the behavioral data (Dews and Weisel, '70) which showed that monocular eyelid suture from birth to 20 days post-partum had no distinguishable effects. Occlusion after 20 days however, resulted in behavioral and electrophysiological anomalies with severity proportional to the length of deprivation.

The occlusions were accomplished by abrading the leading edges of the upper and lower eyelids and then suturing the edges together with 5-0 silk. All surgery was under Halothane anesthesia.

Histology

Before sacrificing Group I and III kittens had their eyes cyclopleged and refracted. All animals were then perfused with 0.9 per cent saline and the brains were removed.

TABLE 1

EXPERIMENTAL PARADIGM

Deprivation Period	Group I Right Eyelid Suture (Occluded Group)	Age at Sacrifice	Group II Right Eyelid Suture with Later Reversal (Reversal Group)	Age at Sacrifice	Group III Normal (Control Group)	Age at Sacrifice
Birth-20 Days	2	20	1	50	1	20
20-30 Days	2	30	1	60	1	30
20-40 Days	2	40	1	70	1	40
20-50 Days	2	50	1	80	1	50
20-60 Days	2	60	1	90	1	60
	N=10		N=5		N=5	

The gyral patterns of each brain were classified according to the four categories of Otsuka and Hassler ('62), the length of the brain measured, and three coronal slices taken starting from the occipital pole. Each slice was 13 per cent of total brain length (4.5-5.0mm thick). The first slice (area 17) and the second slice (areas 17, 18, 19 and ectosylvian gyrus) were processed by the Ramon-Moliner ('64) modification of the Golgi-Cox technique, embedded in celloidin, sectioned at 150$_{\gamma}$ and mounted on slides. The third slice (areas 17, 18, 19) was immersed in ten per cent formalin, embedded in celloidin, sectioned at 35$_{\gamma}$ and stained with cresyl violet. At this point all tissue blocks were number coded to insure a blind study. The code was revealed only at the time of computer analysis.

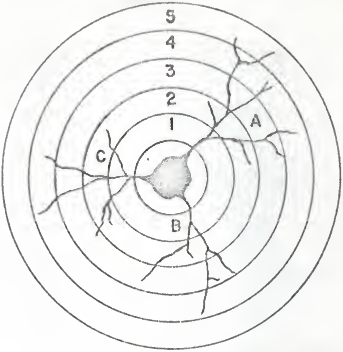
With the tissue stained by the Moliner method every other 150$_{\gamma}$ section was used in the analysis. The number of dendritic spines was counted on a 50$_{\gamma}$ section of pyramidal cell apical dendrite passing through layer IV (stellate cell layer) in the cortex. The spines of nine dendrites (per hemisphere, per section) were counted on neurons whose cell bodies were readily discernable and whose processes could be traced to layer II. The first 4.5-5.0mm slice (occipital pole) contained only area 17. Counts from the second 4.5-5.0mm tissue slice were made in all three visual areas (17, 18, 19). A total of 7,920 apical dendrites were analyzed. All counts were made under oil emersion at 950X. On each cell that apical dendritic spines were counted, a 25μ section on a basal dendrite was also analyzed for the number of spines. As an interlitter control, 22 pyramidal cell apical dendrites in the ectosylvian gyrus of each animal were analyzed.

In order to investigate dendritic branching of stellate cells a modification of the Sholl analysis (Globus and Scheibel, '67) was used. Concentric circles, 620mm apart, were drawn on a sheet of clear plastic and taped to a rear projection screen. The screen was placed 23 inches from a Leitz image projector mounted on a Leitz microscope; this produced an image with a magnification of 635X. The image of a stellate cell perikaryon from layer IV in the visual cortex was centered in the innermost circle or shell (Fig. 1A). Three primary dendrites were selected for analysis. For each shell a dendrite entered, it received a value point of one (Fig. 1B). If it branched once while in a shell, it received a value of two or if it branched again while in a shell, a value of three was given for that shell. The number used for statistical analysis was the total number of all value points received by the three primary dendrites of a given cell. The analysis was done on 48 stellate cells per brain or a total of 960 cells.

Neurons in the tissue stained with cresyl violet were measured for both nuclear size and cortical thickness. On every fourth section, nuclei, with prominent nucleoli, belonging to nine stellate cells in layer IV in each hemisphere were measured (three each from areas 17, 18, 19) using an ocular micrometer. Nuclear volume was determined by the method of Haddara ('56), ($V=4/3\pi ab^2$, where a is one-half the nuclear length and b is one-half the nuclear width). In addition, cell body volume was determined (Haddara, '56) on 36 cells in layer IV from each brain or a total of 660 cells. The plasmo-nuclear ratio could then be determined for cortical neurons following normal and light deprived visual states. Cortical thickness was measured by projecting sections onto white paper and measuring cortical thickness in the five different locations in each hemisphere (Fig. 2) with a vernier caliper.

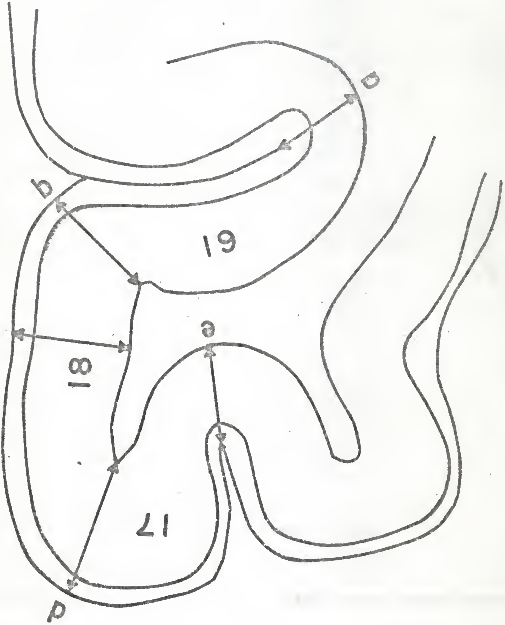
Figure 1 - Top Modified Sholl method used for analysis of dendritic branching.

Bottom Scoring procedure used in analysis of dendritic branching. A, B and C represent the three dendrites of a layer IV stellate cell.



	SHELLS							
		1	2	3	4	5	6	T
DENDRITES	A	2	5	4	5	2		18
	B	2	4	4	1			11
	C	3	5	3	2			13
								42

Figure 2 - Location of the five points of measurement for cortical thickness. Counting sites are indicated by arrows. The numbers 17, 18 and 19 represent the cortical visual areas in the cat.


a
b
19
c
e
18
17
d

RESULTS

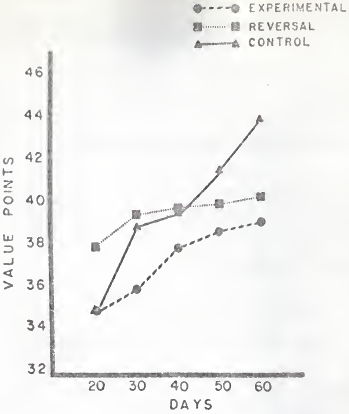
DENDRITIC BRANCHING

The total number of value points (Sholl analysis) received by each of the 960 layer IV stellate cells was used in the analysis (analysis of variance was used throughout the study). There were no statistically distinguishable differences ($P > .05$) between the left and right hemispheres or between area 17, 18 and 19 within groups or between groups.

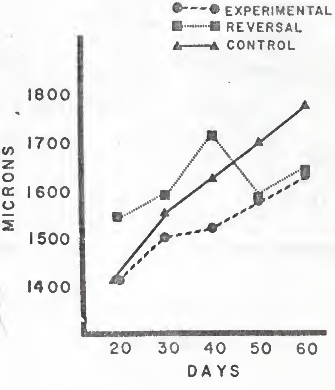
Significant differences ($P < .05$) were found when the experimental control and reversal group means were compared over all sites and both hemispheres (Table 2, Appendix). When days were plotted (abscissa) against Sholl value points (ordinate) these differences were apparent (Fig. 3A). A comparison of the nonoccluded (control) and experimental groups showed a significantly greater amount of dendritic branching in the cortex of the control kittens at all days except day 20 ($P < .05$). Differences were also found between the control and reversal groups. The reversal group showed more branching at day 20 but less at day 60, because of a

Figure 3 - A. Results of Sholl analysis of stellate cell dendritic branching. Higher number of value points indicates greater dendritic branching. Experimental (Group I) kittens had their right eyes occluded for the time periods indicated on abscissa and were then sacrificed. Reversal (Group II) kittens had their right eyes occluded for the time periods indicated and then had that eye opened and the left eyelid sutured for an additional 30 day period whereupon they were sacrificed. Control (Group III) kittens were unoperated normals and were sacrificed along with their Group I counterparts.

B. Results of analysis of cortical thickness. Explanation of groups same as Fig. 3A


EXPERIMENTAL
REVERSAL
CONTROL
VALUE POINTS
46
44
42
40
38
36
34
32
20
30
40
50
60
DAYS

A


EXPERIMENTAL
REVERSAL
CONTROL
MICRONS
1800
1700
1600
1500
1400
20
30
40
50
60
DAYS

B

day-group interaction effect. In the case of the reversal and experimental groups there was a difference in dendritic branching through day 40. In all three groups the amount of branching increased with time. Photomicrographs of experimental and control stellate cells at day 20, 40 and 60 are shown in Fig. 4. The control group cells, as confirmed by the statistical analysis, have longer primary branches and more secondary and tertiary branches.

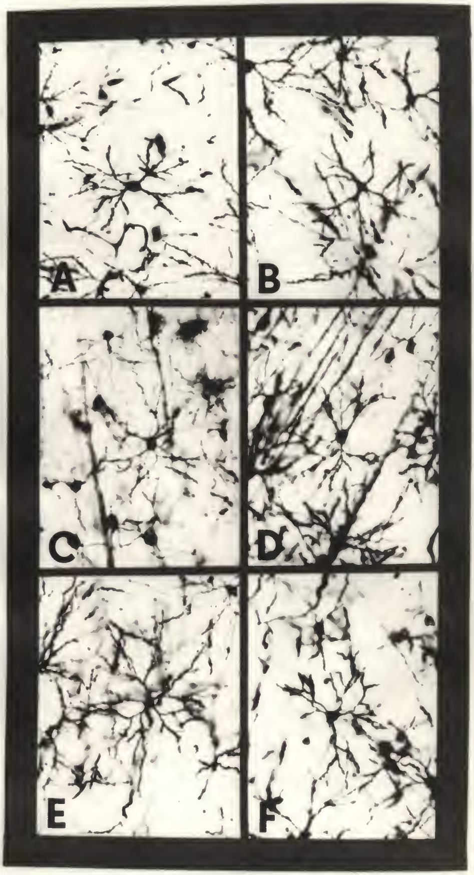
Cortical Thickness

There were statistically significant differences (P .05) between the experimental, control and reversal groups (Table 3, Appendix and Fig. 3B). The control group had a greater average cortical thickness than did the experimental group at all days except day 20 ($P<.05$). The reversal group had thicker visual cortices than either the experimental or control groups until 40 days of deprivation. After this time, the reversal group had thinner cortices than the control group and was statistically indistinguishable from the experimental group ($P>.05$).

Apical Dendritic Spines

There were no statistically distinguishable ($P>.05$) differences in the number of apical dendritic spines when the left and right cortices were compared or when areas 17,

Figure 4 - Photomicrograph of stellate cells in layer IV of visual cortex. Control animals are on the left and the occluded group is on the right. A and B are 20 day cells, C and D are 40 day cells and E and F are 60 day cells.


A
B
C
D
E
F

18 and 19 were compared within groups or between groups. As Fig. 5A shows there were significant overall group differences ($P<.05$). The number of spines in the experimental group was significantly less ($P<.05$) than the number of spines in either the control or reversal groups (Fig. 5A). A comparison of the control and reversal groups showed that although the reversal animals initially had a greater number of dendritic spines there was a greater number of spines after day 40 on the control group neurons. All three groups exhibited an increase in spine density over time. The apical dendritic spines in the experimental and control cortices at days 20, 40 and 60 are shown in Fig. 6. Note that at day 20 there was no significant difference in the number of spines.

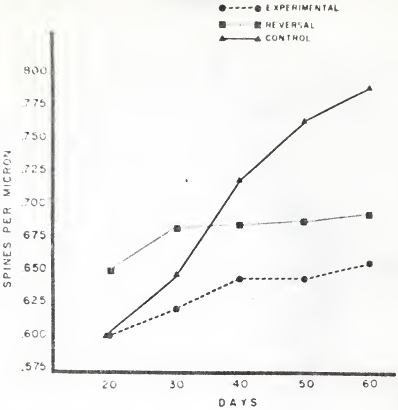
Statistical analysis on neurons from the ectosylvian gyrus, was used as an interlitter control. Unlike areas 17, 18 and 19 neurons in the ectosylvian gyrus showed no statistical distinguishable differences ($P>.05$) between the three experimental groups (control, occluded and reversal).

Basal Dendritic Spines

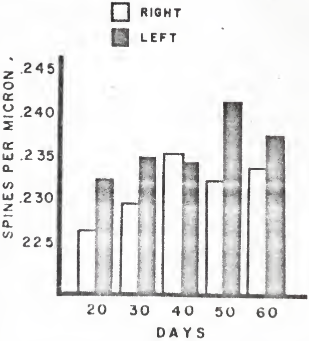
When the experimental, control and reversal groups were compared over all areas (17, 18 and 19) and both hemispheres, there were no statistically significant differences ($P>.05$) in the number of spines on basal dendrites.

Figure 5 - A. Results of apical dendritic spine count. Ordinate gives number of spines per linear micron. Experimental (Group I) kittens had their right eyes occluded for the time periods indicated on abscissa and were then sacrificed. Reversal (Group II) kittens had their right eyes occluded for the time periods indicated and then had that eye opened and the left eyelid sutured for an additional 30 day period whereupon they were sacrificed. Control (Group III) kittens were unoperated normals and were sacrificed along with their Group I counterparts.

B. Results of analysis of basal dendritic spines between left and right hemisphere. Ordinate gives the number of spines per linear micron. These results are across all three groups.


EXPERIMENTAL
REVERSAL
CONTROL
SPINES PER MICRON
.800
.775
.750
.725
.700
.675
.650
.625
.600
.575
20
30
40
50
60
DAYS

A


RIGHT
LEFT
SPINES PER MICRON
.245
.240
.235
.230
.225
20
30
40
50
60
DAYS

B

Figure 6 - Photomicrograph of apical dendritic spines. Control animals are on the left and the occluded group is on the right. A and B are 20 day cells, C and D are 40 day cells and E and F are 60 day cells.

A
B
C
D
E
F

However, a difference was found between the left and right hemispheres with regard to area 17. In all three groups area 17 neurons in the right hemisphere had significantly fewer spines ($P<.05$) on their basal dendrites than did neurons in the left hemisphere (Fig. 5B). No such differences were noted within areas 18 and 19.

Cell Volumes

No significant differences were found between any of the three groups with regard to volume of nuclei, cell body volume or the ratio of nuclei to cell body (Table 2, Appendix).

Refractive Errors

Only the experimental and control animals were refracted before sacrificing. The number of diopters to correction was the unit of analysis. Both groups of animals were found to be hypermetropic or farsighted which is the normal state for kittens. When the difference scores of the left and right eyes of the occluded and control animals were compared, it was found that the difference between the difference scores was significant at $P<.05$ (Table 4, Appendix). This means that while both eyes of normal kittens were isometropic or equal in accomoditive power the eyes of occluded kittens were not.

DISCUSSION

The results indicate that there are demonstrable changes in the kitten visual cortex following monocular deprivation. Some of the changes parallel those previously reported in mice, rats and cats while others do not.

Dendritic Branching

The Sholl method for comparing dendritic branching does not reveal in which of two dimensions the control group was superior to the occluded group. When counting value points for each cell both the length of a dendrite and its ramifications are given equal weight and are not separated. The present results do reveal however, that on one or both of these aspects the occlusion had a deleterious effect. The results are in agreement with the findings of Coleman and Reisen ('66) which showed that visual deprivation decreased dendritic branching by as much as 22 per cent. Hollaway ('67), who had similar findings with impoverished rats, suggests that this may be part of the reason for deprived rats having thinner cortices (Rosensweig, '66). Globus and Schiebel ('67) claimed that deprivation does not cause any real decrease in dendritic branching but that it does cause an increase in variability of dendritic branching. They stated that in some cases the deprived group branching was greater and in some cases less than the controls. In the present study this great variability was not found. Indeed it was the lack of high variability which caused the differences between means to be significant.

The slopes of the curves in Fig. 3A indicate that even with deprivation, dendritic branching increased with age to 60 days after birth. Purpura, Shafer, Housepain and Noback ('64) and Scheibel and Scheibel ('64) also found this to be the case in kittens. The latter investigators found that this maturational increase can continue through 90 days post-partum. The fact that dendritic branching did increase with age partially accounts for the fact that in the cat neuron density falls off from 3130 to 660 per 0.01mm and Von Economo's coefficient (ratio of cell bodies to total gray matter) increases from 9.7 in the newborn kitten to 184 in the adult cat (Brizee and Jacobs, '59).

The interpretation of the reversal group's data was much more complex. The original reasoning behind the reversed suture was that occluding the left eye after opening the right eye would bring about forced use of the previously deprived eye and possibly produce some hemispheric changes which were different from the monocular right eye occluded group. In actuality this did not happen. The reversal group's initially higher scores are most probably due to maturation rather than experimental manipulation since all reversal animals were 30 days older (the recovery time) than their comparison counterparts. When the reversal animals were compared with their chronological agemates in the occluded group (a 20 day reversal kitten with a 50 day occluded kitten), there was no statistical difference ($P > .05$). The most feasible explanation for the lack of any hemispheric difference is the 60-40 ratio of crossed to uncrossed fibers. The only effect the reversed occlusion had was to significantly increase the effects of the earlier monocular occlusion. This fact has some consequences for occlusion therapy for amblyopes. Burian ('66) has questioned this type of therapy when it was found that in many humans the normal eye, when occluded, showed severe loss of acuity and central fixation.

The question arises as to why the occluded group has decreased branching of stellate dendrites. It is known that occlusion brings about deficits in pattern vision (Reisen and Aarons, '59; Reisen, '61; Ganz, Fitch and Satterberg, '69; Von Noorden and Dowling, '70). It seems logical that the ability to discriminate pattern and orientation is partially dependent on inhibition. It has been proposed by Colonnier ('66) that the basket cells of layer IV with their horizontally oriented axons are physiologically similar to the basket cells of the cerebellum and hippocampus. They inhibit the cells lateral to the vertical columns which are formed by the double bouquet cells and pyramidal apical dendrites. Thus, these stellate cells preserve the integrity of the physiological columns found by Hubel and Weisel ('65). Ganz, Fitch and Satterberg ('69) have shown that cortical cells which discharge following stimulation of a deprived eye have unusually large receptive fields and decreased orientation and direction specificity. It would appear that the loss of meaningful, patterned afferent input resulted in a reduction in the growth and spread of the stellate dendrites which were the site of afferent termination. This retarded growth could thus result in decreased visual acuity since the inhibitory or sharpening mechanism functions inadequately.

Cortical Thickness

Care was taken when measuring cortical thickness to get a sampling of gyral shapes. As Bok (cited in Blinkov and Glezer, '68) pointed out the most important consideration when measuring cortical thickness is the location of measurements i.e., apex of gyrus, wall of gyrus, bottom of fissure. In this study five sites were always measured in order to include a wide variety of relief characteristics.

The five per cent difference found between occluded and control cortices was statistically significant (P<.01). Since cell body size does not vary with deprivation the reason for greater thickness in control animals was most probably due to one/all or a combination of the following factors: increased dendritic branching, increases in size and numbers of neuroglial cells, and/or increased vascularity. The present study has shown greater dendritic branching in nondeprived animals. Neuroglial proliferation and increased vascularity were not studied but nontheless some pertinent facts can be gleaned from the literature. Diamond, Law, Rhodes, Lindner, Rosensweig, Krech and Bennett ('66) found a higher neuroglial/neuron ratio in enriched rats than in impoverished rats. Most of the increase was due to oligodendroglia. These authors proffered the hypothesis that the enriched rats had a greater number of neuronal processes and therefore the nutritional needs of the nerve cells were greater. The work of Blinkov and Glezer ('68) showed that there was indeed a correlation between growth of nerve cell processes and neuroglial cells. During ontogenesis, in cats, the neuronal field was increased 20 times and the neuroglial/neuron index increased five times. During this period, the cell body size remained fairly stable.

Vascularity is another factor which undoubtably accounts for increased cortical thickness. The length and number of capillaries increased with the number of synapses and dendritic branching in the cat cortex (Dunning and Wolf, '37). In both of these dimensions the control cats in the present study were superior to the occluded animals. Klosovski and Kosmarskaya (cited in Blinkov and Glezer, '68) found that the blood supply of neurons was unrelated to cell body size but was dependent on functional properties or the neuron's capacity for work. From all the literature previously cited it can be assumed that the control animals exhibit this greater cortical functioning.

Fig. 3B demonstrates that for the normal and occluded groups there was increased cortical thickness with time. The natural growth and maturation of cell processes, neuroglial cells, and blood vessels could account for this increase but does not explain the reversal group data. As with dendritic branching the higher initial scores could be explained by the fact that the reversal group was 30 days older than the other two groups and when chronological age was considered they were not actually different from their occluded group counterparts (a 20 day reversal and a 50 day occluded). From day 40, however, the results were more complex. At day 40 the cortical thickness of the reversal group was maximal and then, it dropped to the same levels as the occluded group at days 50 and 60. This drop means that animals who were actually 80 and 90 days old had the same cortical thickness as 50 and 60 day old animals. The only explanation that can be offered is that the growth and maturation of the developed cortex were arrested because of the alternate occlusion in the reversal group.

Apical Dendritic Spines

When spine counts were done on pyramidal cell dendrites care was taken with respect to the location of the counted area. This precaution was necessary because Valverde ('77) and Marin-Padilla ('67) have shown that the number of spines increased exponentially with distance from the cell body. For the sake of uniformity all counts in the present study were made 150_{γ} away from the pyramidal cell perikaryon.

The present findings show a ten per cent decrease in spines when the occluded group was compared to the control group. This statistically significant decrease confirmed the findings of Valverde ('67) and Fifkova ('71) who also found decreased spines in light deprived mice and rats. These data are contrary to the findings of Globus and Scheibel ('67) who showed that enucleation in rabbits resulted in actual loss of spines and that deprivation brings about only deformity. Although deformity of spines was not analyzed in the present experiment it can be seen from Fig. 6 that deprived animals do show one of the characteristics enumerated by Globus and Scheibel ('67), i.e., lack of end knobs. It may be that this "sick" (Globus and Scheibel, '67) appearance of the spines could be one of the early phases leading up to the actual denuding of the apical shaft or perhaps deformity of spines is the end point of deprivation in rabbits.

Since the present study as well as earlier studies have shown significant changes in stellate cell branching and also numbers of apical dendritic spines, it appears that both stellate and pyramidal cells are primary sites of termination for the geniculo-striate fibers. In the past it was generally held (O'Leary, '41) that direct geniculo-striate input went solely to area 17 where it ended on the stellate cells of layer IV. It was assumed that the stellate cells in area 17 then transferred information to the pyramidal cells. These cells presumably relayed information to areas 18 and 19. More recently, however, Sprague ('65) and Berkley, Wolf and Glickstein ('67) have shown both anatomically and electrophysiologically that in the cat direct geniculate innervation also goes to area 18 and possibly 19. Thus it appears as if all the visual areas get direct projections and that the input terminates on dendrites of both types of cells. As Globus and Scheibel ('67) state, "the stellate cells are lashed in parallel rather than in series to the primary afferent fibers-cortical pyramid sequence."

The interpretation of the reversal group results is the same as was given for dendritic branching; namely that this reversal group was always 30 days more mature than the occluded group. When the two groups were compared at the same chronological age there was no real difference. In order to check on the recovery of spines after varying periods of deprivation another study must be done in which both eyes remain open during recovery.

Basal Dendritic Spines

As Table 2, Appendix shows, there were no differences between reversal, control and occluded groups in the number of spines on the basal dendrites. The spines in this study averaged 0.232 spines per linear μ. The number of spines in rabbits was slightly higher (Globus and Scheibel, '67) with the average being 0.3 spines per μ. Globus and Scheibel ('67), proposed that it is only the main apical shaft that receives specific afferent input. The oblique branches of the dendrite receive only callosal fibers and the basal branches receive fibers from recurrent pyramidal axons and the axons of stellate cells. The present study indicates that environmental manipulation has no effect on basal dendrites but does affect dendrites receiving primary afferent input.

However, there was a statistically significant difference ($P < .05$) in number of basal dendritic spines between hemisphere

The left hemisphere in all animals, regardless of experimental condition, always had significantly more spines on the basal dendrites. This was true, however, only with regard to area 17. In contrast, areas 18 and 19 did not show any hemispheric difference. There may be an anatomical laterality in the visual system. Although this morphological finding has not been previously reported with regard to vision, King (personal communication) has evidence for hemispheric dominance in the auditory system.

Nuclear and Cellular Dimensions

The method originally proposed to measure cell growth with the cresyl violet stain was to measure nuclear volume. Bok ('36) and Bonin ('39) recommended this measure because of the somewhat irregular shape of cell bodies and the imprecise nature of their borders. According to Bok, the cell body could be estimated by the formula $C = N\ 3/2$ where C is the volume of the cell and N the volume of the nucleus. When the nuclei were measured there were no statistical differences among any of the three groups. In consideration of the possibility that the cytoplasm could change without the nuclei changing, the volume of the whole perikaryon was measured. Comparisons between groups again showed no differences. Most of the studies carried out on mice and rats (Gyllenstein, Malmfors and Norrlin, '65, '68; Diamond, '67) have found a difference between deprived and nondeprived animals. In cats, however, Hubel and Weisel ('63) only found cell body changes in LGN but not in the cortex. Perhaps the differences in results were due to species differences. In the cat, unlike the mouse or rat, variability in cortical cells was reflected only in cell processes rather than perikaryon. Blinkov and Glezer ('68) point out that in the rodent, neuronal field only doubles in size during ontogenesis while in cat it increases over 20-fold.

In order to ascertain if there was a relationship between nuclear diameter and cell body size, a ratio of the two was determined. The ratio found was 1:1.47. This ratio was higher than Donaldson's ('24) ratio of 1:1.27 in rats and lower than the ratio obtained from Bok's ('36) general formula which would be approximately 1:1.8. Although the formula varied with species there appears to be a constant ratio between nuclear size and perikaryon size. Even in studies where changes were found between deprived and nondeprived animals there was still a strict relationship between nuclear and cell body size. For example, Diamond ('67) found that there was a 16.2 per cent difference in nuclear size between enriched and impoverished rats and at the same time a 16.7 per cent difference in cell body size.

Refractive Errors

Monocular deprivation in kittens appears to effect the development of the accomodative system. The control kittens, although farsighted, had relatively equal refractive errors in the two eyes while the occluded kittens had a significant difference in errors between the left and right eyes. This asymmetry would have been predicted by van Alphen ('61) because he holds that for normal development of accomodation there must be an operative input and feedback system working during ontogenesis. It may be that the occlusion in the present study somehow hampered this.

General Comments

This paper has shown that the visual system in the cat was amenable to morphological alteration. Perhaps just as important it has been demonstrated that the deprivation necessary to bring this about be only monocular. Intuitively it would seem that if the system was going to be affected at all, enucleation would be the most effective method with binocular deprivation being next. It was known that enucleation was indeed a very effective means of bringing about morphological change. With this method not only was the system denied light and pattern but it was also denied the basic trophic constituents of an intact nervous system.

The question of monocular versus binocular occlusion is more complex. Weisel and Hubel ('65) used the logic that if occluding one eye brought about abnormal electrophysiological effects occluding both eyes should bring about even more drastic changes. They found that this was not the case. It appears at least with cat, that monocular deprivation causes more dramatic effects than binocular deprivation. Guillery ('72) showed that in the LGN the most effective way to bring about anatomical changes through deprivation is to bring about an imbalance in a bilaterally competitive system. In other words, they have found anatomically what Weisel and Hubel ('65) have found electrophysiologically. Guillery ('72) proposes that the visual system is a delicately balanced bilateral system and that with binocular deprivation you are merely subtracting a constant from both sides, leaving the system with absolutely less input, but with the same relative balance in each channel. The more pronounced effects of deprivation can only be achieved when the two input channels which are competing for synaptic sites are thrown into a disequilibrium.

CONCLUSION

In the kitten the developing visual system was modified by manipulating its input. The electrophysiological and behavioral changes previously reported in the literature do have anatomical correlates. Monocular deprivation, accomplished by eyelid suture, brought about decreased dendritic branching of layer IV stellate cells, decreased numbers of apical dendritic spines, decreased cortical thickness and an asymmetry in accommodation. These changes occurred in all three visual areas, 17, 18 and 19 and in both hemispheres. Occlusion did not effect nuclear or cell volume as in rodents and did not have any effect on the spines located on the basal dendrites of pyramidal cells.

The susceptibility of the visual system to deprivation was time dependent. Before 20 days post-partum the development of cortical cells appear to be genotypically controlled and impervious to change from external manipulation. This finding agrees with the results of Hubel and Weisel ('70) which show that there were no electrophysiological changes before this time. After 20 days cell morphology was altered by occlusion. The amount of alteration was directly related to the length of the deprivation period. No strict upper limit of critical period was found in the present study. At 60 days post-partum the gap between the deprived and nondeprived animals was still widening. Although deprivation did have a profound effect on development it still must be noted that in all three groups there was growth in dendritic branching, spine growth and growth of cortex with time.

The study has raised several problems as to the effects of deprivation. It is still a matter of controversy whether the critical period has an upper limit. Fifkova ('71) found that in rats it does not. It is also imperative to find out what characteristic of deprivation is important to what part of the visual system. In all the studies which have been done there have been no successful attempts at exclusive deprivation of pattern vision. In enucleation and occlusion studies both light and pattern are diminished. It may be that general light deprivation affects one part of the system while pattern deprivation affects another component. Weisel and Hubel ('63) were cognizant of this when they deprived cats by both lid closure and occluders. Lid closure cuts down light by four to five log units, while occluders attenuate light by one or two log units. The occluded animals appeared to have a more normal LGN than did the sutured group but damage was the same to the cortices. Diffuse light seems to affect LGN while pattern affects cortex. Further studies are needed before any final conclusions can be drawn with regard to what input elements are necessary for proper cortical development.

APPENDIX

TABLE 2

SUMMARY TABLE OF MEANS

Analysis Over All Sites and Both Hemispheres

	Apical Dendritic Spines Per μ	Dendritic Branching (value points)	Basal Dendritic Spines Per μ	Volume of Nuclei (μ^3)	Volume of Cell Body (μ^3)	Nuclei Cell Body Ratio
Control	.705*	39.88*	.231	782.6	977.4	1:1.41
Reversal	.673*	38.55*	.233	779.7	969.0	1:1.44
Experimental	.633*	37.34*	.232	782.6	974.3	1:1.43
Total Number Cells	7,920	960	7,960	10,800	660	660

* F values for group x day interaction significant at .05 level

TABLE 3

MEASUREMENTS OF CORTICAL THICKNESS IN MICRONS

	Site 1	Site 2	Site 3	Site 4	Site 5	Average
Control	1371.84	1900.20	1767.17	1535.33	1528.70	1620.64*
Reversal	1411.04	1882.94	1756.22	1585.85	1500.64	1621.35*
Experimental	1274.49	1816.08	1697.09	1468.36	1453.26	1542.85*

* F value for group x day interaction significant at .01 level

TABLE 4

MEAN DIFFERENCE SCORES OF DIOPTERS TO CORRECTION

Experimental	Control
4.437*	2.26*

*F values significant at .05 level

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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